

Application No.: 09/529,217

Docket No.: 21029-00196-US

AMENDMENTS TO THE CLAIMS

1. (currently amended) A method of qualitative and quantitative analysis of microbial population(s) comprising:

providing a sample containing microorganisms,

contacting the microorganisms present in the sample with at least one specific probe to form a sample with a probe-target complex using *in situ* hybridization in whole cells, wherein the specific probe recognizes a RNA target sequence,

contacting the sample with the probe target complex with a wash solution to remove excess specific probes or non-specific probes from the sample with the probe target complex thereby providing a washed sample.

adding a denaturing agent to the washed sample [extracting the hybridized] to extract the specific probes from the probe-target complex [in the contacted sample by adding a denaturing agent to denature the probe-target complex], and

detecting the extracted probes and measuring the amount thereof or their respective amounts to provide the qualitative and quantitative analysis of the microorganisms in the sample.

2. (previously presented) A method according to Claim 1, wherein said at least one specific probe is chosen among the group consisting of Nb 1000 (SEQ ID N°1).

3. (previously presented) A method according to Claim 1, further comprising contacting said microorganisms present in said sample with an universal probe to normalize results.

4. (previously presented) A method according to Claim 3, wherein said universal probe is chosen among the group consisting of S Univ-1390 SEQ ID N°3) and S Bac 338 (SEQ ID N°4).

5. (previously presented) A method according to Claim 3 wherein said specific or said universal probe is a mRNA-targeted probe.

Application No.: 09/529,217

Docket No.: 21029-00196-US

6. (previously presented) A method according to Claim 1, further comprising extracting said microorganisms in said sample by centrifugation.

7. (currently amended) A method according to Claim 1, [wherein said] further comprising fixing of said whole cells prior to contacting [is performed following fixation of said whole cells] the microorganisms with the at least one specific probe.

8. (previously presented) A method according to Claim 7, wherein fixation of the cells is achieved by incubation of the cells in a fixation solution of less than 10% paraformaldehyde for 3 to 12 hours at 4°C.

9. (currently amended) A method according to Claim 7, wherein said fixation is followed by a dehydration step, prior to said contacting [step] the microorganisms with the at least one specific probe.

10. (previously presented) A method according to Claim 9, wherein the dehydration is performed by placing said sample in contact with at least one ethanol solution.

11. (currently amended) A method according to Claim 1, wherein said contacting the microorganisms with the at least one specific probe includes [is performed by] placing said sample in contact with said specific probe in the presence of a hybridization solution comprising a denaturing agent at a concentration of from 0.001% to 0.1%, Tris-HCl with a pH of about 8 at a concentration of from 0.001 M to 0.1 M; and a salt at a concentration of from 0.1 M to 1.5 M.

12. (currently amended) A method according to Claim 1, wherein contacting the microorganisms with the at least one specific probe includes [is performed for] an incubation time of about 10 minutes to about 2 hours, and at an optimal hybridization temperature.

13. (currently amended) A method according to claim 1, wherein [extracting of said specific probe is performed following removal of excess and unbound specific probe or of non-specifically associated probe material by contacting with a] the wash solution [comprising]

Application No.: 09/529,217

Docket No.: 21029-00196-US

comprises a denaturing agent and a salt at concentrations appropriate for achieving the stringency necessary for the removal of non-specifically associated probe.

14. (previously presented) A method according to Claim 1, wherein extracting of the hybridized probes includes extracting at a temperature higher than the melting temperature of the specific probe under consideration.

15. (currently amended) A method according to Claim 14, wherein extracting of the hybridized probes includes adding [the denaturing agent is] formamide to the washed sample.

16. (previously presented) A method according to Claim 1, wherein said extracted probes are concentrated prior to the measurement of the amount thereof or of their respective amounts.

17. (currently amended) A method according to Claim 1, wherein said detecting and measuring the amount of the extracted probes includes detection and quantification of a label associated with or incorporated into the extracted probes, wherein the label is selected from a radioactive label, a chemiluminescent label or a fluorescent label.

18. (previously presented) A method according to claim 1, wherein said sample is taken from fluids selected from natural water, industrial water, industrial effluent, municipal wastewater, industrial sludge, thermal mud, food liquid or gel, fermentation media, air, gas, aerosol, a sample taken from a building ventilation duct or air conditioning duct, a sample of food solid, a sample of soil, a sample from medical apparatus, or is a human or animal sample selected from blood, urine, vaginal or intestinal flora.

19. (previously presented) A method according to Claim 1, wherein said method is used in combination with a process for triggering an alarm in connection with quality, safety and or sanitary monitoring of the product from which said sample has been obtained.

Application No.: 09/529,217

Docket No.: 21029-00196-US

20. (currently amended) A method according to Claim 1, wherein [it] said method is used in *in vitro* diagnosis of an infectious disease.

21. (currently amended) A method according to Claim 1, wherein [it] said method is used in the automatic or feedback control of a microbiological process such as methane fermentation of liquid manure, treatment of organic effluents, sewage treatment process such as treatment by activated sludge.

22. (currently amended) A method according to Claim 1, wherein [it] said method is used in the automatic or feedback control of a process relating to the removal or prevention of the development of microorganisms.

23. (currently amended) A method according to Claim 1 wherein [it] said method is applied in the detection of foam formation during the implementation of activated sludge processes and/or the feedback control of a method relating to the removal or prevention of the said foams.

24. (previously presented) A method according to claim 10, wherein the dehydration comprises a series of ethanol solutions of increasing concentrations.

25. (currently amended) A method according to claim 11, wherein the concentration of said denaturing agent [concentration is on the order of] is about 0.01%, the concentration of said Tris-HCl [concentration is on the order of] is about 0.02 M, and the concentration of said salt [concentration is on the order of] about 0.9 M.

26. (previously presented) A method according to claim 11, wherein said denaturing agent is sodium dodecyl sulfate and said salt is sodium chloride.

27. (previously presented) A method according to claim 13, wherein said denaturing agent is sodium dodecyl sulfate and said salt is sodium chloride.

Application No.: 09/529,217

Docket No.: 21029-00196-US

28. (previously presented) A method according to claim 17, wherein said label is fluorescein.

29. (previously presented) A method according to claim 8, wherein said fixation solution contains about 4% paraformaldehyde.

30. (previously presented) A method according to claim 14, wherein said denaturing agent comprises formamide.